

Measurement by Electron Paramagnetic Resonance (EPR) Spectrometry of ^{15}N -Labelled Nitrosothiols in Human Plasma and Gastric Juice Following the Ingestion of ^{15}N -Labelled Inorganic Nitrate

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Ingested nitrate from the diet is concentrated in human saliva as nitrite, before swallowing and reacting with gastric acid to form nitrous acid [1]. We set out to test if protein/peptide nitrosothiols (RSNOs) are formed in the gastric juice and plasma after nitrate ingestion.

Healthy volunteers ($n=5$) ingested 2mmol ^{15}N -labelled sodium nitrate ($\text{Na}^{15}\text{NO}_3$). Paired gastric juice and plasma samples were obtained at 15-minute intervals from the time of ingestion ($t=0$) until $t=90$ minutes. The samples were analysed by EPR spectrometry in the presence of iron *N*-methyl *D*-glucamine dithiocarbamate ($(\text{MGD})_2\text{-Fe}^{2+}$). The two EPR signals, centred on $g=2.04$ mT, were distinguished by their hyperfine splitting – $(\text{MGD})_2\text{-Fe}^{2+}\text{-NO}$ had a triplet signal ($a_{\text{N}}=1.28$ mT) while $(\text{MGD})_2\text{-Fe}^{2+}\text{-}^{15}\text{NO}$ had a doublet signal ($a_{^{15}\text{N}}=1.90$ mT).

$(\text{MGD})_2\text{-Fe}^{2+}\text{-}^{15}\text{NO}$ was undetectable in all of the gastric juice and plasma samples at $t=0$. The gastric juice RS^{15}NO concentration was significantly increased to $1.71 \pm 5.52\mu\text{M}$, median \pm interquartile range (IQR), by 30 minutes ($p<0.05$), with a maximum at 90 minutes ($21.57\mu\text{M}$; $p<0.05$), whilst the plasma RS^{15}NO concentration was significantly increased to $51.16 \pm 122.56\text{nM}$ by 90 minutes ($p<0.05$). The plasma RS^{15}NO concentrations were consistently lower than those of the gastric juice samples.

Our data shows an increase in RS^{15}NO in plasma following the ingestion of ^{15}N -labelled nitrate, indicating that this is due to the passage of nitrous acid or its metabolites from the gastric juice to the plasma.

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[1] Duncan C *et al. Nat.Med.* 1, 546-51, 1995